Protein Structure Prediction & Drug Design

2377-Pos
Searching for Novel HIV-1 Reverse Transcriptase Inhibitors: a Relaxed Complex Approach
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Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) currently represents the fourth leading cause of death worldwide and has claimed the lives of over 25 million people since 1981. To combat this infectious disease, a wide range of drugs has been discovered, inhibiting various stages of the retroviral life cycle. The most popular target is the HIV-1 reverse transcriptase (RT) enzyme, which is required to convert retroviral RNA into DNA, and for which substantial high-resolution structural data exist. Here, we focus on the non-nucleoside RT inhibitor (NNRTI) class of compounds, which confer high specificity and block DNA polymerization in an allosteric fashion. Despite the FDA-approval of four NNRTIs to date, the side-effects of drug toxicity and the emergence of drug-resistance mutations demand further drug discovery endeavors. To this end, we have performed a virtual screening study of the RT enzyme, with the aim of discovering novel NNRTI lead compounds from the National Cancer Institute (NCI) library. To take into account the conformational flexibility of the protein, we have screened mini-libraries of NCI compounds against diverse ensembles of RT structures. Firstly, we use a traditional, experimental source of structures: x-ray crystallography. Next, in an effort to expose novel conformations of the binding site that might be missed experimentally, we use a theoretical source: molecular dynamics (MD) simulation. To achieve this, we have carried out all-atom, explicit solvent MD simulation of the RT enzyme, complexed with a variety of NNRTIs. By integrating the results from the crystallographic and MD ("relaxed") ensemble screens, we compile a set of the most promising candidate compounds for experimental testing.

2378-Pos
Recreating the Structure and Dynamics of Ancestral Proteins
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Although protein structures are more evolutionarily conserved than protein sequences, the majority of work done in studying mutations in evolutionarily diverged proteins is at the DNA sequence level. In this work, we use a combination of the Zipping Assembly Method of protein structure prediction (1) and FRODA, a constrained geometric Monte Carlo simulation (2) used for exploring conformational space, to reconstruct ancestral proteins. By using reservoir Replica Exchange Molecular Dynamics we effectively both determine the native like conformation and sample near native states (3). We then dock the appropriate ligands to the predicted native ancestral structure and near native conformations to compute both ligand binding affinity and also binding poses. Initial results from simulations of ~440 million year old ancestral glucocorticoid and corticoid steroid receptor proteins indicate that we are able to fold these proteins to <3Å from native and that our calculated ligand binding affinities agree with experimental results (4) from reconstructed ancestral proteins. Furthermore, by examining the flexibility of ancestral proteins, we can identify which mutations are most critical in shifting binding affinity from promiscuous binding to highly specific binding of only a few ligands. This also gives insight into the non-reversibility of evolution and into the design of new molecules.
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2379-Pos
Computational Methods for Mapping Ligand Induced Conformational Changes in GPCRs
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The active conformation of a G-Protein Coupled Receptor (GPCR) is influenced by the chemical structure and efficacy of the bound ligand. Insight into the active conformation and activation pathway for ligands with different efficacies is critical in designing functionally specific drugs for GPCRs. Using the computational method LITiCon, we have predicted the activation pathways for full, partial and inverse agonists of β2 Adrenergic Receptor, which are in agreement with fluorescence intensity lifetime measurements. Starting from the inactive state, the full agonists take the receptor to a stable intermediate in an energy downhill step (fast step), followed by a barrier crossing leading to the active state (slow step). The reasons for the energy barrier are the disruption of a HB between N293 on TM6 and S204 on TM5 as well as the one between D113 on TM3 and the β-hydroxyl group on the ligand. During MD simulation of norepinephrine bound β2-AR, water polarization helps to disrupt the HB between D113 and β-hydroxyl thus facilitating the barrier crossing. In contrast with the full agonists, the activation pathway for the partial agonist salbutamol involves only a slow step, which stems from steric interactions between the ligand and the aromatic residues on TM6.
We have further refined the LITiCon method to optimize the backbone tilts and kinks of the transmembrane helices in the presence of a bound ligand. We will present the details of the method and the results on validation of rhodopsin activation by all-trans retinal, and β2-AR bound to various agonists to investigate the modulation of helical kinks and tilts due to agonist binding.

2380-Pos
Homology Modelling and Molecular Dynamics Simulations of the TAP ABC Transporter
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ATP-Binding Cassette (ABC) transporters constitute one of the largest families of membrane proteins. These proteins transport a variety of substrates and are associated with a large number of diseases such as multi-drug resistance in cancer, diabetes, cystic fibrosis, and immune deficiencies. Several members of the ABC super-family act as a pump to import or export molecules; others are lipid flippases or regulatory subunits of potassium channels, and one member acts as a chloride channel. Despite this functional diversity, ABC transporters share a common mechanism of action, using energy derived from ATP hydrolysis to allow the translocation of their substrates. The basic architecture consists of two transmembrane domains (TMDs) coupled with two nucleotide binding domains (NBDs), where the binding and the hydrolysis of ATP drives specific conformational changes, in turn transmitted to TMDs to complete the transport cycle. The transporter associated with antigen processing (TAP) is an ABC heterodimer (TAP1/TAP2) which translocates peptides into the ER for loading them onto MHC class I molecules. Using the crystal structures of P-glycoprotein (1) in an inward-facing conformation and of Sav1866 in outward-facing conformation (2), we built homology models of TAP corresponding to the initial and final stages of the transport cycle, respectively. We carried out molecular dynamics simulations of these models to investigate the structural features of the different conformational states of TAP, as a fundamental step to elucidate the mechanism of action of this transporter.
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2381-Pos
High-Performance Drug Discovery: Computational Screening by Combining Docking and Molecular Dynamics Simulations
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Lead discovery is one of the most important processes in rational drug design. To improve the rate of the detection of lead compounds, various technologies such as high-throughput screening and combinatorial chemistry have been introduced into the pharmaceutical industry. However, since these technologies alone may not improve lead productivity, computational screening has become important. A central method for computational screening is molecular docking. This method generally docks many flexible ligands to a rigid protein and predicts the binding affinity for each ligand in a practical time. However, its ability to detect lead compounds is less reliable. In contrast, molecular dynamics simulations can treat both proteins and ligands in a flexible manner, directly estimate the effect of explicit water molecules, and provide more accurate binding affinity, although their computational cost and time are significantly greater than those of molecular docking. Therefore, we developed a special purpose computer "MDGRAPE-3" for molecular dynamics simulations and applied it to computational screening. In this paper, we report an effective method for computational screening; this method is a combination of molecular docking and massive-scale molecular dynamics simulations. The proposed method showed a higher and more stable enrichment performance than the molecular docking method used alone.